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REVIEW OF KALINA'S BOOK ON DIRECTED MODIFICATION OF BACTERIA

/The following is taken from a review of a book by G. P. Kalina entitled Vegetativnaya Gibridizatsiya i Napravlennaya Izmenchivost' Bakteriy (Vegetative Hybridization and the Directed Modification of Bacteria), State Medical Ukrainian Publishing House, Kiev, 1952, 283 pp./

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In his book, Kalina pays particular attention to the phenomenon of para-agglutination. He regards para-agglutination as an indication of an initial reorganization of the antigenic apparatus of microorganisms that is connected with a modification of hereditary properties, i e , as a sign that transformation into another species is beginning to take place He discusses in great detail the conditions under which parastrains form in individual intestinal infections and under different experimental conditions. The author equates the concept of a parastrain with that of a vegetative hybrid He proposes that the properties newly acquired and exhibited by the parastrain be referred to as paraproperties, parareactions, and parametabolism. He uses the designation of "mentor" for microbes which confer their properties to other microbiological species, basing this designation on Michurin's terminology.

In discussing the effect of the macroorganism on the formation of paraproperties, Kalina assumes that the immune substances of the macroorganism probably are of importance in the formation of these properties. He remarks with sufficient justification that formation of parastrains takes place when the bacteria which acquire the new properties exist together with bacteria which transmit these properties, or are exposed to the action of the metabolism products of the bacteria which transmit these properties

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The author shares the view that the protoplasm of the microbial cell is a single giant molecule which has a well-expressed structure and a unified organization of cellular protein that exhibits a structure of the micelle type. He rejects the primitive ideas according to which bacteria possess a semipermeable membrane through which metabolism proceeds in conformity with the laws of osmosis and diffusion. On the basis of contemporary data pertaining to the metabolism of the bacterial cell, he comes to the conclusion that all metabolic processes take place on the surface of this cell, the so-called ectoplasm. This active surface of the cell is not an indifferent semipermeable membrane, according to Kalina. Kalina assumes that the protoplasm transmits impulses from the outside into its depth, so that the total contents of the cell are involved in metabolic processes. From this standpoint, the absorption of enzymes from the external medium under subsequent stimulation of the synthesis of identical enzymes by the cell can be explained. Analogously to enzymes, other colloidal substances (proteins and proteids) can be adsorbed and assimilated by the cell. This assumption permits the explanation of the presence in freshly isolated bacteria of antigenic structures common with the organism from which these bacteria have been isolated.

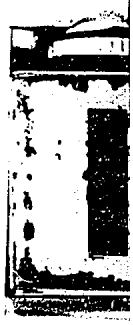
The action of bacteriophages is regarded by the author as a two-stage process. The first stage is adsorption of the phage by the multiplying cell and the second is assimilation of the phage nucleoproteid and modification of the way in which cell nucleoproteids are synthesized.

Basing his conclusions on the existence of penetration of some organized forms of living matter into others (e. g., multiplication of the virus of tobacco mosaic and interaction of lactic acid bacteria or of their filterable forms with yeast cells), Kalina thinks it is extremely probable that high-molecular substances are assimilated by the surface of bacterial cells just as described above. Going one step further, he assumes the possibility of assimilation by bacteria of filterable forms originating from other bacteria. In the reviewer's opinion, this assumption does not contribute much to clarification of the phenomenon of transformation into other species, because the term "filterable forms" is rather indefinite, covering as it does many biologically diverse particles. The properties of these particles even differ depending on the type of filter and on the conditions of filtration.

However, Kalina's view that the total cell structure is modified by the assimilation of any proteins at the surface is undoubtedly correct. The modifications brought about in this manner constitute the very essence of vegetative hybridization.

Kalina has studied complete antigens and nucleoproteids as agents which are likely to bring about species modifications in bacteria. While his experiments with antigens led to negative results, those with nucleoproteids were successful. He succeeded in proving the effectiveness of the nucleoproteid component of dysentery bacilli in transforming B. coli. According to Kalina's data, the phenomenon of para-agglutination could be observed after two reseedings of B. coli into a medium containing the nucleoproteid in question.

However, Kalina's further discussion of results obtained in experiments with nucleoproteids leads to confusion. He asserts that he obtained filterable forms of bacteria from the nucleoproteids and that these filterable forms had the capacity of developing again into the original bacterial culture from which the nucleoproteids were originally obtained. It is clear that the nucleoproteids must have contained live bacteria, possibly in a filterable form. The customary technique of preparing nucleoproteids does not provide for sterile conditions. A similar situation must have existed in connection with the author's experiments on the irradiation of dysentery bacilli with ultraviolet rays.



Identification of filterable forms of bacteria with nucleoproteids (cf p 275 of Kalina's book) may lead to far-reaching conclusions, and must therefore be based on a more substantial array of experimental data. Filterable forms of bacteria, including those of bacteria of the intestinal group under discussion at present, consist of live protoplasm which is capable of metabolism, although this metabolism may not be developed as fully as in complete bacteria. It is yet to be shown that nucleoproteids exhibit metabolism.

The role of filterable forms of bacteria in the formation of hybrid variations cannot be disputed at present. What remains to be established is the exact mechanism of the interaction between various species of microbes or between their filterable forms. However, it is hardly to be assumed that hybridization takes place as a result of direct penetration into microbes of filterable forms which have a pronounced capacity to regenerate spontaneously the full-valued initial cultures from which they have been derived. That the filterable forms are capable of undergoing this regeneration has been established by many experiments performed by workers at Kalina's laboratory [Laboratory of the Medical Institute, Chernovtsy, according to an article in the Trudy (Works) of the Institute of Microbiology, Academy of Sciences USSR, Issue 1, 1951, p 60]. According to data obtained by these workers, regeneration of filterable forms may occur without the influence of factors that exert a specifically directing effect on metabolism, if conditions exist which contribute to the appearance and development of recessive characteristics pertaining to the initial species of microbes. Such conditions exist if cultivation is carried out in media containing blood, bile, or some other suitable ingredients. It is necessary to recognize that the process of regeneration may be modified as a result of the assimilation of metabolites which are foreign to the species of the microbes being regenerated. These metabolites may originate from another species of microbes, which then exerts a directing influence in changing the original characteristics of the species that has given rise to the filterable forms.

Filterable forms, being more pliable, have unstable hereditary characteristics, as witnessed by the suppressed activity of the first generation of regenerated cultures. They can be brought out of the inert state by means of specific biochemical agents and they then show modified characteristics if they have been subjected to directed modification. In other words, they become hybrid variations or parastrains. To explain the formation of such strains, it is not at all necessary to assume penetration of microbial cells by filterable forms.

After exhaustively discussing the subject of para-agglutination, the author turns to the problem of directed modification. He does not devote enough space to this problem, which he treats on four pages only. Kalina defines directed modification and distinguishes between several types of it, differing in regard to the degree of modification. He states that the modifications may range from purposely limited changes of one or two characteristics, in such a manner that the microorganism still remains within the original species, to thoroughgoing changes which constitute transformation into another species.

According to Kalina, the first kind of modification may involve stable loss of virulence with preservation of immunogenic properties (this is the type of modification which leads to the formation of live vaccines), adaptation to a definite type of nutrition, directed antagonism, and breeding for pathogenic properties ("vospitaniye patogenosti"). The second kind of modification, according to Kalina, involves multiple adaptation. He states that in the course of multiple adaptation, the modification is not limited to the characteristic which is directly connected with the mode of action on the microorganism, but also involves other characteristics. As a result, properties arise which are typical for species that normally exist under the conditions brought about artificially in order to modify the initial microorganism. This is the type of change which results in the transformation of one species of microorganism into another.

Kalina is firmly convinced that transformation of microbiological species into other species takes place under natural conditions and calls the attention of epidemiologists to the significance of this transformation.

In addition to the general remarks made above, the reviewer considers it necessary to discuss individual parts of Kalina's monograph which require specific criticism. First of all, the facility with which the author obtains experimental results is well-nigh astonishing. For example, he observed development of paraproperties after only two reseedings into a medium containing nucleoproteids; he was able to isolate parastrains from 50-70% of dysentery patients (according to one claim, even in 100% of the cases) and 44-80% of typhoid patients; he succeeded in isolating filterable forms from vaccines heated to 100°; he regularly obtained regeneration of filterable forms contained in the blood and organs of rabbits which had been immunized with various vaccines; he observed a 100% para-agglutination of B. coli colonies after they had been grown for 2 days in a 2- to 3-day-old culture of dysentery bacilli; etc. Kalina's claim that he achieved transformation of paracoli bacilli into a dysentery strain by passing it only once through Bactoagar Zh is surprising; in view of the fact that Bactoagar Zh suppresses the growth of ordinary intestinal bacteria like B. paracoli, one must assume that he started with a mixed culture of dysentery and paracoli bacilli.

Kalina's statement that the controls were sterile in all cases except for the possible presence of filterable forms does not contribute to a clearer understanding of the complex problems of modifiability. The assertion that active transformation has taken place is not quite convincing in the light of the author's experimental results to the effect that filterable forms survive a temperature of 100° and that they regenerate the initial bacterial form with exceptional facility.

Kalina uses the term "hybridization" rather freely. The analogy which he draws between microorganisms and higher plants is not quite successful. He does not treat with sufficient clarity the problem of dissociation. In Kalina's opinion, dissociation cannot be regarded as modification in the true sense of the word.

The author regards as particularly valuable the work done by foreign investigators in contributing to a more precise definition of interphase dissociative transitions. This indicates that he has a tendency to defend the theory of cyclogeny. Without any comments or reservations, he refers to the regularity and conformity with natural law of the transition from the R-phase to the S-phase.

It seems to the reviewer that the materialistic concept that dissociation constitutes adaptive modification within the limits of the species, and that the changes resulting from it enable the microbes to survive under suboptimal conditions has been adequately confirmed and should be accepted without any reservations.

In general, one may say that Kalina's book presents interesting and detailed factual data which have a bearing on the modification of microorganisms, and that the author's intention to treat the facts from the standpoint of Michurinist biology has been successfully realized. However, there are many vague interpretations and some errors, so that one must read the book with a critical attitude.

Kalina's book can hardly be recommended as a standard handbook or as a textbook for beginners. However, it touches on many problems which are of great practical value and of considerable importance in contemporary microbiology. The creative effort of Soviet microbiologists should be applied to the solution of these problems.

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